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N-Pantoyl-(substituted)amines, Pantothenic Acid Analogues

JOHN D. FISSEKIS, CHARLES G. SKINNER and WILLIAM SHIVE,
Clayton Foundation Biochemical Institute and the Department of Chemistry, The University of Texas, Austin, Texas

Introduction

The synthesis of inhibitory analogues of vitamins and other metabolites has been widely used as a method of obtaining potential pharmaceutically active compounds. Effective analogues of pantothenic acid have been obtained through modification of either the pantoyl (α,γ-dihydroxy-β,β-dimethylbutyryl-) radical or the β-alanine portion of the compound.[1] Several *N*-pantoylalkylamines and related compounds have been prepared and found to inhibit the growth of various micro-organisms[2,3] and in most instances the toxicities were competitively reversed by pantothenic acid. In an effort to examine further the effect of varying the structure of the β-alanine moiety in this manner, some additional analogues of the vitamin were prepared by interacting pantolactone with arylalkylamines. The inhibitory properties of these compounds varied with the test system used, but several were found to be more inhibitory than the previously reported *N*-pantoylalkylamines.

Experimental*

MICROBIOLOGICAL ASSAYS

A previously reported[4] assay medium was used for the studies with *Lactobacillus arabinosus* 17–5 and *Streptococcus lactis* 8039,

* The ultraviolet spectra of certain compounds were studied in a Beckman DK–2 ratio recording spectrophotometer, and/or a Beckman DU spectrophotometer. All melting points are uncorrected. The chromatograms were obtained using the ascending technique in the solvents indicated.

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except that a solution containing 17 g per litre of commercial casein hydrolysate* was used instead of the acid hydrolysed casein as indicated in the reference, and the concentration of the vitamin supplement was increased three-fold. For *Leuconostoc mesenteroides* P–60 the above modified medium was used except that in addition the Salts A concentration was increased four-fold. The medium was supplemented with D(+) calcium pantothenate as indicated in the tables.

The assay tubes were incubated at 30° for about 17 h unless otherwise indicated in the tables. The amount of growth was determined turbidimetrically in terms of galvanometer readings so adjusted that in the particular instrument distilled water read 0 and an opaque object 100.

Organic Syntheses

Amines. Unless otherwise stated, the amines were obtained through normal commercial sources and were redistilled, and the refractive indices measured to establish purity prior to condensing with pantolactone. ω-Phenylpropyl-, butyl, and amylamines, as well as 2-α-naphthylethylamine, were prepared as previously reported.[5] ω-Phenylheptylamine and ω-phenoxybutylamine were also prepared by a previously reported procedure;[6] however, the boiling point of the former derivative was erroneously reported through a typographical error in the cited reference, and should have been 174–177°/20 mm.†

Picrolonate Salts of Amines. The amines used in this study were further characterized by preparing the corresponding picrolonates which are summarized below. All of these derivatives were prepared by interacting a few drops of the corresponding amine with a half-saturated ethanol solution of picrolonic acid. In most instances the derivative crystallized from the solution after standing overnight in the refrigerator; otherwise, the reaction mixture was warmed over a steam cone for a few minutes prior to placing it in a refrigerator to induce crystallization.

* Nutritional Biochemicals Corporation, Cleveland, Ohio.

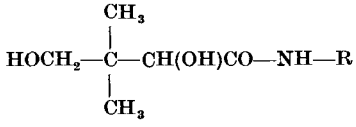
† Braun, J. v. *Ber.* **44**, 2867 (1911) reported a b.p. of 159–160°/16 mm.

Amine used, R—NH_2	m.p., °C (d.)	Empirical formula	Analysis, % N	
			Calcd.	Found
2-Phenylethyl-	212–213	$C_{18}H_{19}N_5O_5$	18·17	17·97
3-Phenylpropyl-	208–209	$C_{19}H_{21}N_5O_5$[a]	17·54	17·52
5-Phenylpentyl-	159–160	$C_{21}H_{25}N_5O_5$	16·38	16·73
7-Phenylheptyl-	148–149	$C_{23}H_{29}N_5O_5$[b]	15·38	15·13
4-Phenoxybutyl-	182–183	$C_{20}H_{23}N_5O_6$	16·31	16·11
2-α-Naphthylethyl-	228–229	$C_{22}H_{21}N_5O_5$	16·08	15·87
3-Methoxypropyl-	181–182	$C_{14}H_{19}N_5O_6$[c]	19·83	19·72
2-Pyridyl-	265–266	$C_{15}H_{14}N_6O_5$	23·46	23·31

[a] Calcd: C, 57·13; H, 5·30. Found: C, 57·22; H, 5·50.
[b] Calcd: C, 60·64; H, 6·42. Found: C, 60·55; H, 6·36.
[c] Calcd: C, 47·59; H, 5·42. Found: C, 47·49; H, 5·71.

N-*Pantoyl-(substituted)amines.* The syntheses of these compounds were patterned on a previously reported procedure,[2,3] and the experimental data are summarized in Table I. A mixture of equivalent amounts of the corresponding amine and pantolactone were heated at 100° for about 2 h in a sealed tube. For the lower boiling amines, the reaction mixture was heated *in vacuo* for about 6 h to remove the unreacted material, and the residue was then taken up in ether. With the higher boiling amines, the reaction mixture from the sealed bombs was taken up in ether, and the ether phase was subsequently washed twice with 10 per cent hydrochloric acid, then water, then twice with 10 per cent sodium carbonate solution, and finally water. The resulting ether solution from both procedures was dried over sodium sulphate, treated with Norit, filtered, reduced in volume, and Skellysolve B was added to induce turbidity. Several of the products crystallized from these solutions after standing for a long period of time. Crystallization of an oil could also be induced by adding a crystal of a homologue of the compound when available. The solid product once obtained could be easily recrystallized from ether-Skellysolve B. In three instances, a crystalline product could not be obtained; however, the oil gave an acceptable elemental analysis for the anticipated compound as indicated in Table I.

Table I. *N*-Pantoyl-(substituted)amines



R—	Isolation technique[a]	m.p., °C (d.)	Empirical formula	Analysis, %					
				Calcd.			Found		
				C	H	N	C	H	N
3-Phenylpropyl-	D	[b]	$C_{15}H_{23}NO_3$			5·28			5·35
4-Phenylbutyl-	D	72–74	$C_{16}H_{25}NO_3$	68·78	9·02	5·01	68·85	9·01	5·10
5-Phenylpentyl-	E	63–64	$C_{17}H_{27}NO_3$	69·59	9·28	4·77	69·46	9·10	4·83
7-Phenylheptyl-	E	[b]	$C_{19}H_{31}NO_3$			4·36			4·27
4-Phenoxybutyl-	D	72–73	$C_{16}H_{25}NO_4$	65·06	8·53	4·74	65·23	8·44	4·75
2-α-Naphthylethyl-	D	96–98	$C_{18}H_{23}NO_3$	71·73	7·69	4·64	71·84	7·84	4·64
3-Methoxypropyl-	D	[b]	$C_{10}H_{21}NO_4$			6·39			6·31
2-Pyridyl-	D	51–53	$C_{11}H_{16}N_2O_3$			12·49			12·32

[a] D–Purified by distillation.
E–Purified by extraction.

[b] Material isolated and analysed as an oil.

Each of the condensation products was examined by paper chromatographic techniques in several solvent systems, and all were found to be homogeneous with respect to inhibiting growth in bioautographs.

N-*Pantoylcarboxymethoxylamine*(N-*Pantoylaminoöxyacetic Acid*). To a solution of sodium (50 mg) in methanol (4·6 ml) carboxymethoxylamine hemihydrochloride* (183 mg) was added, after which, pantolactone (261 mg) dissolved in methanol (3 ml) was added, and the reaction mixture was heated under reflux for about 8 h. The resulting solution was cooled in an ice bath, adjusted to pH 2 with concentrated hydrochloric acid, and taken to dryness *in vacuo*. After the addition and evaporation of a few millilitres of additional methanol, the resulting residue was chromatographed and found to be free of unreacted aminoöxyacetic acid. The solid was then treated with 3 ml of ethanol, the inorganic salts were removed by filtration, and the filtrate was again reduced to dryness *in vacuo* to yield an oily residue. The semi-solid material was dissolved in water (5 ml), and the acidic solution was adjusted to pH 6·5 by addition of powdered cinchonine with efficient stirring. The resulting insoluble material was filtered, washed with cold water, and the combined filtrates were reduced to dryness *in vacuo*. The crystalline residue was washed with cold acetone : ether (1 : 4 by volume), and finally with ether alone to yield 496 mg of product, which after recrystallization from acetone-Skellysolve B gave white needles, m.p. 216° (d.)

Anal. Calcd. for $C_8H_{15}NO_6 . 1·5\ C_{19}H_{22}N_2O$: C, 66·14; H, 7·30; N, 8·45. Found: C, 66·38; H, 7·30; N, 8·21.

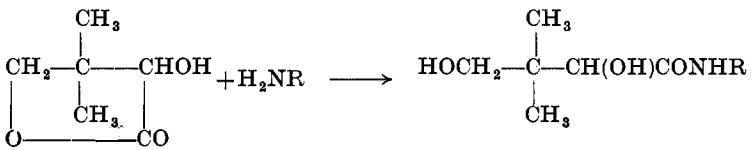
The unexpected molecular ratio between the *N*-pantoylaminoöxyacetic acid and cinchonine was substantiated by comparing the ultraviolet absorption spectra of free cinchonine with that of the cinchonine salt condensation product.

Results and Discussion

Several *N*-pantoylamine derivatives were readily prepared by a thermal condensation of equimolar mixtures of the appropriate

* Aldrich Chemical Co., Milwaukee, Wisconsin.

amine and DL-pantolactone as indicated in the accompanying equation.



The major synthesis problem with these derivatives involved crystallizing the resulting oily residue from the several condensations. In only one instance did a crystalline material result directly from the condensation, and that was in the case of the 2-α-naphthylethylamine derivative. More frequently the semi-solid residue was alternately dissolved and precipitated from ether-Skellysolve B solutions with intermittent cooling and seeding until the mass began to crystallize. Once a crystalline mass was obtained, the solid material could then be recrystallized from a suitable solvent with relative ease. In three instances, with 3-phenylpropylamine, 7-phenylheptylamine and 3-methoxypropylamine, a crystalline derivative was not obtained; however, the semi-solid material recovered from the reaction mixture gave an acceptable elemental analysis for the anticipated derivative.

Almost all the pantothenic acid analogues reported in this investigation were inhibitory in at least one of the micro-organisms at a relatively low concentration. As a basis of comparison of biological activity with the previously reported *N*-pantoyl-(substituted)amines, *N*-pantoyl-*n*-butylamine was assayed in the several microbiological systems as a control.

For *Leuconostoc mesenteroides* P–60, the most inhibitory pantothenic acid analogue tested was *N*-pantoyl-7-phenylheptylamine, which had an inhibition index of 2·5 for maximal inhibition; *N*-pantoylbutylamine had an inhibition index of 5 under these assay conditions. The data for the other analogues prepared and tested are reported in Table II, and their inhibition indices for maximal inhibition varied from 25 to 250 in this organism. Of the ten *N*-pantoyl-(substituted) amines studied in *Lactobacillus arabinosus* 17–5, the most inhibitory analogue was also *N*-pantoyl-7-phenylheptylamine which had an inhibition index for maximal inhibition of about 400. The analogues in general were

Table II. Comparative susceptibility of various organisms to inhibition by pantothenic acid analogues

Inhibitor	Molar Ratios (Analogue to Pantothenic Acid) for Indicated Inhibition of Organism					
	Leuconostoc mesenteroides P–60[a]		*L. arabinosus* 17–5[b]		*S. lactis* 8039[b]	
	Half maximum	Maximum	Half maximum	Maximum	Half maximum	Maximum
DL-*N*-Pantoyl-*n*-butylamine	0·7	5	400	2,000	—	—
DL-*N*-Pantoyl-3-phenylpropylamine	4	25	300	2,000	550	1,000
DL-*N*-Pantoyl-4-phenylbutylamine	11·2	50	200	1,000	470	1,000
DL-*N*-Pantoyl-5-phenylpentylamine	5	25	300	1,000	25	50
DL-*N*-Pantoyl-7-phenylheptylamine	0·56	2·5	175	400	180	500
DL-*N*-Pantoyl-4-phenoxybutylamine	8·5	50	600	2,000	100	200
DL-*N*-Pantoyl(2-α-naphthylethyl)amine	45	250	350	2,000	200	1,000
DL-*N*-Pantoyl(3-methoxypropyl)amine	7	50	350	2,000	—	—

[a] Incubated at 30° for 30 h.

[b] Incubated at 30° for 17 h.

appreciably less inhibitory in this organism than in *Leuconostoc mesenteroides* as indicated in Table II. For *Streptococcus lactis* 8039, the most toxic derivative was *N*-pantoyl-5-phenylpentylamine which had an inhibition index for maximal inhibition of 50; the values for the other derivatives studied varied between 200 and 1000. In the latter two micro-organisms, *N*-pantoylbutylamine was appreciably less inhibitory than the two cited analogues.

N-Pantoylaminoöxyacetic acid did not have sufficient toxic properties to carry out a microbiological study in any of the three organisms used in this study.

The degree of biological activity of the various *N*-pantoyl-(substituted)amines in the different micro-organisms suggests that *Leuconostoc mesenteroides* and *L. arabinosus*, have enzyme sites for utilization of pantothenic acid which are structurally different from the site of action of the natural metabolite in *S. lactis*. A structural specificity for each organism is thus indicated which is dependent upon the length of the carbon chain attached to the pantoyl grouping. The effect of the introduction of various active functional groups in the ω-phenyl radical would be of interest, since such a grouping might irreversibly combine with the active enzyme sites in the host and thus produce a more potent inhibitor. An irreversible combination would produce an antagonist which would not be competitively reversed by pantothenic acid.

Table III. Reversal of toxicity of *N*-pantoyl-7-phenylheptylamine by D(+)calcium pantothenate in *Leuconostoc mesenteroides* P-60[a]

N-Pantoylphenylheptylamine, μg/ml	Supplement: D(+)Calcium Pantothenate, μg/ml			
	0·04	0·08	0·16	0·32
	Galvanometer readings			
0	37	58	63	72
0·01	28	49		
0·02	21	41	47	
0·05	6	16	29	69
0·1		6	19	60
0·2			8	43
0·4				5

[a] Incubated at 30° for 30 h.

Where possible, all of the analogues presented in this study were examined in each of the three micro-organisms for their ability to be reversed by D(+)calcium pantothenate. The data obtained for the most inhibitory compound in each assay system are presented in Tables III, IV and V. The low solubility of

Table IV. Reversal of toxicity of *N*-pantoyl-7-phenylheptylamine by D(+)calcium pantothenate in *Lactobacillus arabinosus* 17–5[a]

N-Pantoylphenyl-heptylamine, μg/ml	Supplement: D(+)Calcium Pantothenate, μg/ml			
	0·004	0·008	0·016	0·032
	Galvanometer readings			
0	28	55	74	88
0·2	20			
0·5	12	47		
1·0	8	35	69	
2·0		17	58	84
4·0		7	29	72
8·0			7	35
16·0				7

[a] Incubated at 30° for 17 h.

Table V. Reversal of toxicity of *N*-pantoyl-5-phenylpentylamine by D(+)calcium pantothenate in *Streptococcus lactis* 8039[a]

N-Pantoylphenyl-pentylamine, μg/ml	Supplement: D(+)Calcium Pantothenate, μg/ml			
	0·01	0·02	0·04	0·08
	Galvanometer readings			
0	44	66	59	57
0·1	55			
0·2	49	71		
0·5	21	55	75	
1·0	6	13	63	73
2·0		8	16	67
4·0			6	18
8·0				9

[a] Incubated at 30° for 17 h.

many of these derivatives prevented a more extended range of concentration studies. Where the inhibition was sufficient to carry out a study over a wide range of concentrations, all of the *N*-pantoyl-(substituted)amines presented herein were found to be competitively reversed by pantothenic acid.

Summary. A thermal condensation between pantolactone and various amines has resulted in the formation of nine new *N*-pantoyl-(substituted)-amines. The derivatives crystallized with difficulty; an acceptable elemental analysis, however, could be obtained even on the analogues which were isolated as semi-solids. The condensation product with carboxymethoxylamine (aminoöxyacetic acid) proved to be difficult to purify, and it was finally isolated as a cinchonine salt.

The inhibitory properties of the pantothenic acid analogues were examined in three organisms, *Lactobacillus arabinosus* 17–5, *Streptococcus lactis* 8039, and *Leuconostoc mesenteroides* P–60. For purposes of comparison, the toxicities of the derivatives were related to that of *N*-pantoylbutylamine, a previously reported effective competitive antagonist of pantothenic acid. For *Leuconostoc mesenteroides* and *L. arabinosus*, the most effective competitive antagonist prepared in this study was *N*-pantoyl-7-phenylheptylamine, which had inhibition indices for maximal inhibition of 2·5 and 400, respectively, in these two micro-organisms. In *S. lactis*, the most inhibitory analogue studied was *N*-pantoyl-5-phenylpentylamine which had an inhibition index for maximal inhibition of 50. In every instance cited above the indicated analogue was more toxic than *N*-pantoylbutylamine, and they were competitively reversed by D(+)calcium pantothenate over a significant range of inhibitor concentrations.

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